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L5	l4 and detect$3	1	L5
L4	L3 and (hybridiz$5 near5 probe$1)	1	L4
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L2	phosphonomethylglycine	891	L2
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L6: Entry 1 of 1 File: USPT Oct 8, 2002

DOCUMENT-IDENTIFIER: US 6462258 B1
TITLE: Plant expression constructs

Brief Summary Text (4):
One of the goals of plant genetic engineering is to produce plants with agronomically important characteristics or traits. Recent advances in genetic engineering have provided the requisite tools to produce transgenic plants that contain and express foreign genes (Kahl et al., World J. of Microbiol. Biotech. 11:449-460, 1995). Particularly desirable traits or qualities of interest for plant genetic engineering would include but are not limited to resistance to insects, fungal diseases, and other pests and disease-causing agents, tolerances to herbicides, enhanced stability or shelf-life, yield, environmental tolerances, and nutritional enhancements. The technological advances in plant transformation and regeneration have enabled researchers to take exogenous DNA, such as a gene or genes from a heterologous or a native source, and incorporate the exogenous DNA into the plant's genome. In one approach, expression of a novel gene that is not normally expressed in a particular plant or plant tissue may confer a desired phenotypic effect. In another approach, transcription of a gene or part of a gene in an antisense orientation may produce a desirable effect by preventing or inhibiting expression of an endogenous gene.

Brief Summary Text (5):
In order to produce a transgenic plant, a construct that includes a heterologous gene sequence that confers a desired phenotype when expressed in the plant is introduced into a plant cell. The construct also includes a plant promoter that is operably linked to the heterologous gene sequence, often a promoter not normally associated with the heterologous gene. The construct is then introduced into a plant cell to produce a transformed plant cell, and the transformed plant cell is regenerated into a transgenic plant. The promoter controls expression of the introduced DNA sequence to which the promoter is operably linked and thus affects the desired characteristic conferred by the DNA sequence.

Brief Summary Text (9):
Thus, according to one embodiment of the invention, a recombinant DNA construct is provided that comprises, in operable linkage, a promoter that is functional in a cell of a crop plant, the promoter comprising: at least one cis element derived from SEQ ID NO:12, SEQ ID NO:22, and SEQ ID NO:23, a structural DNA sequence heterologous to the promoter; and a 3' non-translated region that functions in plants to cause the addition of polyadenylated nucleotides to the 3' end of the RNA sequence. For example, the promoter may consist essentially of a 5' regulatory region derived from any of SEQ ID NO:12, SEQ ID NO:22, and SEQ ID NO:23, (including or excluding any intron sequences located therein). The structural gene may comprise any heterologous nucleotide sequence wherein expression of the sequence results in an agronomically useful trait or product in a transgenic crop plant.

Brief Summary Text (12):
According to another embodiment of the invention, DNA constructs, such as those described above, are provided in tandem, wherein the promoter is a hybrid or chimeric promoter comprising at least one cis element derived from one or more of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 operably linked to a heterologous gene sequence that expresses in transgenic crop plant cells. The chimeric promoter sequences more specifically comprising the sequences identified in SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30.

Brief Summary Text (14):
According to another embodiment of the invention, transgenic crop plants are provided that are transformed with a DNA construct as described above, including monocot species and dicot species. We have discovered that the Arabidopsis actin and Arabidopsis EF1.alpha. promoters are sufficiently active in other crop plant species such as cotton, tomato, and sunflower, for example, that when used to control expression of a glyphosate tolerance gene, such as aroA:CP4, the plants tolerate commercial application rates of glyphosate, exhibiting good vegetative tolerance and low damage to reproductive tissues. Such promoters can also be used to express other genes of interest in plants, including, but not limited to, genes that confer herbicide tolerance, insect control, disease resistance, increased stability or shelf, higher yield, nutritional enhancement, expression of a pharmaceutical or other desired polypeptide product, or a desirable change in plant physiology or morphology, and so on.

Brief Summary Text (15):
According to another embodiment of the invention, transgenic crop plants are provided that are transformed with multiple DNA constructs comprising the Arabidopsis actin and Arabidopsis EF1.alpha. promoters are sufficiently active in other plant species such as cotton, tomato, sunflower, for example, that when used to control expression of a glyphosate tolerance gene such as aroA:CP4, the plants tolerated commercial application rates of glyphosate, exhibiting good vegetative tolerance and low damage to reproductive tissues. Such promoters can also be used to express other genes of interest in plants, including, but not limited to, genes that confer herbicide tolerance, insect control, disease resistance, increased stability or shelf, higher yield, nutritional enhancement, expression of a pharmaceutical or other desired polypeptide product, or a desirable change in plant physiology or morphology, and so on.

Brief Summary Text (16):
According to another embodiment of the invention, transgenic crop plants are provided that are transformed with DNA constructs comprising the Arabidopsis actin and Arabidopsis EF1.alpha. promoters as chimeric DNA molecules in fusion with caulimovirus DNA molecules having promoter activity in plants sufficiently active in other plant species such as cotton, tomato, canola, soybean, and sunflower, for example, that when used to control expression of a glyphosate tolerance gene such as aroA:CP4, the plants tolerate commercial application rates of glyphosate, exhibiting good vegetative tolerance and low damage to reproductive tissues. Such promoters can also be used to express other genes of interest in plants, including, but not limited to, genes that confer herbicide tolerance, insect control, disease resistance, increased stability or shelf, higher yield, nutritional enhancement, expression of a pharmaceutical or other desired polypeptide product, or a desirable change in plant physiology or morphology, and so on.

Detailed Description Text (11):
A "recombinant" nucleic acid is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques. Techniques for nucleic-acid manipulation are well-known (see for example Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, 1989; Mailga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Press, 1995; Birren et al., Genome Analysis: volume 1, Analyzing DNA, (1997), volume 2, Detecting Genes, (1998), volume 3, Cloning Systems, (1999) volume 4, Mapping Genomes, (1999), Cold Spring Harbor, N.Y.).

Detailed Description Text (15):
"Transformed", "transfected", or "transgenic" refers to a cell, tissue, organ, or organism into which has been introduced a foreign nucleic acid, such as a recombinant construct. Preferably, the introduced nucleic acid is integrated into the genomic DNA of the recipient cell, tissue, organ or organism such that the introduced nucleic acid is inherited by subsequent progeny. A "transgenic" or "transformed" cell or organism also includes progeny of the cell or organism and progeny produced from a breeding program employing such a "transgenic" plant as a parent in a cross and exhibiting an altered phenotype resulting from the presence of a recombinant construct or construct.

Detailed Description Text (32):
The promoter sequences of the present invention may be modified, for example for expression in other plant systems. In another approach, novel hybrid promoters can be designed or engineered by a number of methods. Many promoters contain upstream sequences which activate, enhance or define the strength and/or specificity of the promoter (Atchison, Ann. Rev. Cell Biol. 4:127, 1988). T-DNA genes, for example contain "TATA" boxes defining the site of transcription initiation and other upstream elements located upstream of the transcription initiation site modulate transcription levels (Gelvin, In: Transgenic Plants (Kung, S.-D. and Us,R., eds, San Diego: Academic Press, pp.49-87, 1988). Another chimeric promoter combined a trimer of the octopine synthase (ocs) activator to the mannopine synthase (mas) activator plus promoter and reported an increase in expression of a reporter gene (Min Ni et al., The Plant Journal 7:661, 1995). The upstream regulatory sequences of the present invention can be used for the construction of such chimeric or hybrid promoters. Methods for construction of variant promoters of the present invention include but are not limited to combining control elements of different promoters or duplicating portions or regions of a promoter (see for example U.S. Pat. Nos. 5,110,732 and 5,097,025). Those of skill in the art are familiar with the specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolation of genes, (see for example Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, 1989; Mailga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Press, 1995; Birren et al., Genome Analysis: volume 1, Analyzing DNA, (1997), volume 2, Detecting Genes, (1998), volume 3, Cloning Systems, (1999) volume 4, Mapping Genomes, (1999), Cold Spring Harbor, N.Y.).

Detailed Description Text (45):
In addition to their use in modulating gene expression, the promoter sequences of the present invention also have utility as probes or primers in nucleic acid hybridization experiments. The nucleic-acid probes and primers of the present invention can hybridize under stringent conditions to a target DNA sequence. The term "stringent hybridization conditions" is defined as conditions under which a probe or primer hybridizes specifically with a-target sequence(s) and not with non-target sequences, as can be determined empirically. The term "stringent conditions" is functionally defined with regard to the hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic-acid sequence of interest) by the specific hybridization procedure (see for example Sambrook et al., 1989, at 9.52-9.55, Sambrook et al., 1989 at 9.47-9.52, 9.56-9.58; Kanehisa, Nucl. Acids Res. 12:203-213, 1984; and Wetmur and Davidson, J. Mol. Biol. 31:349-370, 1968). Appropriate stringency conditions which promote DNA hybridization are, for example, 6.0.times.sodium chloride/sodium citrate (SSC) at about 45.degree. C., followed by a wash of 2.0.times.SSC at 50.degree. C., are known to those skilled in the art or can be found in laboratory manuals including but not limited to Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 1989, 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0.times.SSC at 50.degree. C. to a high stringency of about 0.2.times.SSC at 50.degree. C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22.degree. C., to high stringency conditions at about 65.degree. C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed. For example, hybridization using DNA or RNA probes or primers can be performed at 65.degree. C. in 6.times.SSC, 0.5% SDS, 5.times.Denhardt's, 100 .mu.g/mL nonspecific DNA (e.g., sonicated salmon sperm DNA) with washing at 0.5.times.SSC, 0.5% SDS at 65.degree. C., for high stringency.

Detailed Description Text (46):
A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low stringency" conditions. Similarly, the molecules are said to be "complementary" is they can hybridize to one another with sufficient stability to permit them to remain

annealed to one another under conventional "high stringency" conditions. It is contemplated that lower stringency hybridization conditions such as lower hybridization and/or washing temperatures can be used to identify related sequences having a lower degree of sequence similarity if specificity of binding of the probe or primer to target sequence(s) is preserved. Accordingly, the nucleotide sequences of the present invention can be used for their ability to selectively form duplex molecules with complementary stretches of DNA fragments. Detection of DNA segments via hybridization is well-known to those of skill in the art, and thus depending on the application envisioned, one will desire to employ varying hybridization conditions to achieve varying degrees of selectivity of probe towards target sequence and the method of choice will depend on the desired results. Conventional stringency conditions are described in Sambrook, et al., Molecular Cloning, A Laboratory Manual, 2.sup.nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989, and by Haymes et al., Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C., 1985.

Detailed Description Text (50):
Probes and primers are generally 11 nucleotides or more in length, preferably 18 nucleotides or more, more preferably 25 nucleotides, and most preferably 30 nucleotides or more. Such probes and primers hybridize specifically to a target DNA or RNA sequence under high stringency hybridization conditions and hybridize specifically to a target native sequence of another species under lower stringency conditions. Preferably, probes and primers according to the present invention have complete sequence similarity with the native sequence, although probes differing from the native sequence and that retain the ability to hybridize to target native sequences may be designed by conventional methods. Methods for preparing and using probes and primers are described, for example, in Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989 (hereinafter, "Sambrook et al., 1989"); Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel et al., 1992); and Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. PCR-primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, .COPYRGT. 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Primers and probes based on the native promoter sequences disclosed herein can be used to confirm and, if necessary, to modify the disclosed sequences by conventional methods, e.g., by re-cloning and re-sequencing.

Detailed Description Text (57):
For the practice of the present invention, conventional compositions and methods for preparing and using DNA constructs and host cells are employed, as discussed, inter alia, in Sambrook et al., 1989. In a preferred embodiment, the host cell is a plant cell. A number of DNA constructs suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., Cloning Vectors: A Laboratory Manual, 1985, supp. 1987); Weissbach and Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989; Gelvin et al., Plant Molecular Biology Manual, Kluwer Academic Publishers, 1990; and R.R.D. Croy Plant Molecular Biology LabFax, BIOS Scientific Publishers, 1993. Plant expression constructs can include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences. They can also include a selectable marker as described to select for host cells containing the expression construct. Such plant expression constructs also contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and a polyadenylation signal. Other sequences of bacterial origin are also included to allow the construct to be cloned in a bacterial host. The construct will also typically contain a broad host range prokaryotic origin of replication. In a particularly preferred embodiment, the host cell is a plant cell and the plant expression construct comprises a promoter region as disclosed in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NOS:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30; an operably linked transcribable sequence; and a transcription termination sequence. Other regulatory sequences envisioned as genetic components in an expression construct include but is not limited to non-translated leader sequence which can be

coupled with the promoter. In a particularly preferred embodiment, the host cell is a plant cell and the plant expression construct comprises a promoter region as disclosed in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NOS:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30; an operably linked transcribable sequence, and a transcription termination sequence. Plant expression constructs also can comprise additional sequences including but not limited to polylinker sequences that contain restriction enzyme sites that are useful for cloning purposes.

Detailed Description Text (61):
Plant expression constructs can include RNA processing signals, e.g., introns, which may be positioned upstream or downstream of a polypeptide-encoding sequence in the transgene. In addition, the expression constructs may include additional regulatory sequences from the 3'-untranslated region of plant genes (Thornburg et al., Proc. Natl. Acad. Sci. USA 84:744 (1987); An et al., Plant Cell 1:115 (1989), e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions. 5' non-translated regions of a mRNA can play an important role in translation initiation and can also be a genetic component in a plant expression construct. For example, non-translated 5' leader sequences derived from heat shock protein genes have been demonstrated to enhance gene expression in plants (see, for example U.S. Pat. No. 5,362,865). These additional upstream and downstream regulatory sequences may be derived from a source that is native or heterologous with respect to the other elements present on the expression construct.

Detailed Description Text (70):
Methods for specifically transforming dicots primarily use Agrobacterium tumefaciens. For example, transgenic plants reported include but are not limited to cotton (U.S. Pat. No. 5,004,863; U.S. Pat. No. 5,159,135; U.S. Pat. No. 5,518,908, WO 97/43430), soybean (U.S. Pat. No. 5,569,834; U.S. Pat. No. 5,416,011; McCabe et al., Bio/Technology, 6:923, 1988; Christou et al., Plant Physiol., 87:671, 1988); Brassica (U.S. Pat. No. 5,463,174), and peanut (Cheng et al., Plant Cell Rep., 15: 653, 1996).

Detailed Description Text (71):
Similar methods have been reported in the transformation of monocots. Transformation and plant regeneration using these methods have been described for a number of crops including but not limited to asparagus (Asparagus officinalis; Bytebier et al., Proc. Natl. Acad. Sci. U.S.A., 84: 5345, 1987); barley (Hordeum vulgarae; Wan and Lemaux, Plant Physiol., 104: 37, 1994); maize (Zea mays; Rhodes, C. A., et al., Science, 240: 204, 1988; Gordon-Kamm, et al., Plant Cell, 2: 603, 1990; Fromm, et al., Bio/Technology, 8: 833, 1990; Koziel, et al., Bio/Technology, 11: 194, 1993); oats (Avena sativa; Somers, et al., Bio/Technology, 10: 1589, 1992); orchardgrass (Dactylis glomerata; Horn, et al., Plant Cell Rep., 7: 469, 1988); rice (Oryza sativa, including indica and japonica varieties, Toriyama, et al., Bio/Technology, 6: 10, 1988; Zhang, et al., Plant Cell Rep., 7: 379, 1988; Luo and Wu, Plant Mol. Biol. Rep., 6: 165, 1988; Zhang and Wu, Theor. Appl. Genet., 76: 835, 1988; Christou, et al., Bio/Technology, 9: 957, 1991); sorghum (Sorghum bicolor; Casas, A. M., et al., Proc. Natl. Acad. Sci. U.S.A., 90: 11212, 1993); sugar cane (Saccharum spp.; Bower and Birch, Plant J., 2: 409, 1992); tall fescue (Festuca arundinacea; Wang, Z. Y. et al., Bio/Technology, 10: 691, 1992); turfgrass (Agrostis palustris; Zhong et al., Plant Cell Rep., 13: 1, 1993); wheat (Triticum aestivum; Vasil et al., Bio/Technology, 10: 667, 1992; Weeks T., et al., Plant Physiol., 102: 1077, 1993; Becker, et al., Plant, J. 5: 299, 1994), and alfalfa (Masoud, S. A., et al., Transgen. Res., 5: 313, 1996). It is apparent to those of skill in the art that a number of transformation methodologies can be used and modified for production of stable transgenic plants from any number of target crops of interest.

Detailed Description Text (76):
In one embodiment a greenhouse or field evaluation for glyphosate tolerance is conducted. The term "glyphosate" is used herein to refer collectively to the parent herbicide N-phosphonomethylglycine (otherwise known as glyphosate acid), to a salt or ester thereof, or to a compound which is converted to N-phosphonomethylglycine in plant tissues or which otherwise provides N-phosphonomethylglycine in ionic form (otherwise known as glyphosate ion). Illustratively, water-soluble glyphosate salts useful herein are disclosed in U.S. Pat. No. 3,799,758 and No. 4,405,531 to Franz, the

disclosure of which is incorporated herein by reference. Glyphosate salts that can be used according to the present invention include but are not restricted to alkali metal, for example sodium and potassium, salts; ammonium salt; C.sub.1-16 alkylammonium, for example dimethylammonium and isopropylammonium, salts; C.sub.1-16 alkanolammonium, for example monoethanolammonium, salt; C.sub.1-16 alkylsulfonium, for example trimethylsulfonium, salts; mixtures thereof and the like. The glyphosate acid molecule has three acid sites having different pKa values; accordingly mono-, di- and tribasic salts, or any mixture thereof, or salts of any intermediate level of neutralization, can be used.

Detailed Description Text (131):
The plant expression constructs used for tomato transformation are listed in Table 4. Tomato plants (T0) containing constructs comprising at least one actin or elongation factor promoter (with intron) operably linked to an aroA:CP4 glyphosate tolerance gene are screened in a greenhouse glyphosate spray test with glyphosate (Roundup Ultra.TM.) formulation for the efficiency of conferring glyphosate tolerance to transgenic tomato plants. Optionally, at least one actin or elongation factor promoter sequence operably linked to an aroA:CP4 gene and an eFMV caulimovirus promoter operably linked to an aroA:CP4 transformed into tomato plants are screened by spray application with glyphosate (Roundup Ultra.TM.). Tomato plants are sprayed with 48 oz./acre then evaluated at two weeks post application for analysis of vegetative tolerance and up to 60 days post-application for analysis of reproductive tolerance. The results are shown in Table 4 and ranked according to efficiency of selecting reproductive tolerant lines. The percent vegetative tolerance is the percentage of the lines screened that demonstrated sufficient vegetative tolerance to glyphosate damage to be considered for further studies of agronomic traits in preparation for commercially candidacy. The percent reproductive tolerance is the percentage of the vegetative tolerant lines that also demonstrated sufficient reproductive tolerance to be considered for further agronomic evaluation. All of the constructs proved functional for providing vegetative tolerance and reproductive tolerance to the transgenic tomato plants. Various combinations of promoters are able to increase the efficiency at which vegetative and reproductive tolerant lines could be selected by screening in this experiment. Constructs containing the Arabidopsis EF1.alpha. promoter are more specifically associated with a high percentage of vegetatively tolerant lines. P-Act2 promoter in combination with P-eFMV and P-AtEF1.alpha. (pCGN9190) provided an increase in the percentage of reproductively tolerant lines that are screened by this method.

Detailed Description Text (132):
Tomato seed yield is used as a measure of the efficacy of the various promoter sequences and combination of expression cassettes used in the present invention for conferring glyphosate tolerance to transgenic tomato plants. In Table 5, the results of three field experiments are shown on transgenic tomato plants containing constructs with the promoters of the present invention driving expression of the aroA:CP4 coding sequence for glyphosate tolerance. Experiment 1 is a test of the plants produced from the constructs that contain the Figwort mosaic virus promoter (P-FMV) in the native and the duplicated version (P-eFMV) and additional genetic elements in the constructs that are also found in the constructs used to test the promoter sequences of the present invention. Additional genetic elements such as the source of the 5' untranslated sequence and the chloroplast transit peptide are also tested. The construct pMON20998 comprises the P-eFMV, linked to the petunia Hsp70 5' UTR, leader linked to the Arabidopsis EPSPS chloroplast transit peptide (CTP2), linked to the E9 3' termination region. The construct pMON20999 differs from pMON20998 only in that the promoter is P-FMV. The construct pMON10156 differs from pMON20998 only in that the CTP is from the Petunia EPSPS chloroplast transit peptide (CTP4). The construct pMON45312 differs from pMON20998 only in that the leader sequence is the native FMV leader sequence.

Detailed Description Text (137):
Arabidopsis genomic DNA (100 ng) was used in 50 .mu.l PCR reactions. Reactions containing the primers shown in Table 5. contained 10 .mu.M reverse and forward primer solutions, 200 nM dNTPs and PCR buffer with magnesium and DNA polymerase mix from Expand.TM. High Fidelity PCR System (Roche Molecular Biochemicals). After initial 2 minute denaturation at 94.degree. C. reactions were cycled 0.5 min at 94.degree. C., 0.5 min at 55.degree. C. and 1.5 minute at 72.degree. C. for 35 times. PCR products were analyzed by electrophoresis on 1% agarose gel. Gel isolated DNA fragments

representing Actin 1a, Actin 1b, Actin 7, and Actin 12 sequences were phosphorylated with T4 DNA kinase and ligated to dephosphorylated and Sma I cut pUC19 cloning construct. White colonies were screened for the presence of appropriate inserts and sequenced with M13 forward and reverse primers to confirm the presence of actin promoters. Selected clones were designated as pMON54941 (P-AtAct1a), pMON54942 (P-AtAct1b), pMON54943 (P-AtAct7) and pMON54944 (P-AtAct12). Subsequently, the Actin promoters DNA fragments were released by Hind III and NcoI digest of the pUC19 constructs containing the insert sequences, the DNA fragments were gel isolated and ligated to pMON26165 that had been digested with the same restriction enzymes. A PCR product for the Actin 3 promoter (P-AtAct3) was digested with Hind III and Nco I and cloned directly into pMON26165 to form pMON54951. pMON26165 contains the GUS/nos terminator gene segment. Ligation with the promoter segments allows for assay of each promoter for functional activity by expression of the .beta.-glucuronidase enzyme in plant cells. The plant cells can be isolated, for example, tobacco leaf protoplasts, or the plant cells may be contained in a plant tissue or organ, such as, leaf, root, cotyledon, hypocotyl, embryo, flower, or storage organ. The expression level of GUS driven by these promoters is assayed in soybean hypocotyl in comparison with GUS driven by P-e35S promoter (Table 6). Plasmid DNA/gold particles was bombarded to soybean hypocotyls then after 48 hours the GUS activity was assayed histochemically. All of the Actin promoters tested in this assay show functional activity in the hypocotyl tissue demonstrating their utility for expression transgenes in heterologous crop plant species.

Detailed Description Text (140):
Cotton yield is correlated with the number of squares set during the first four to five weeks of squaring. The retention of these squares to mature bolls and their contribution to the harvest of the cotton lint is a key component of yield. When determining the efficacy of transgene constructs for conferring herbicide tolerance in cotton, the amount of boll retention is a measure of efficacy and is a desirable trait. Transgenic cotton plants containing promoters of the present invention (Table 7) were assayed in greenhouse conditions for boll retention. The promoters directed expression of the aroA:CP4 coding sequence for glyphosate tolerant phenotype. The plants were transformed by an Agrobacterium-mediated method or by a particle gun method. The particle gun constructs contained an additional GUS containing expression cassette useful for histochemical localization of .beta.-glucuronidase activity from the promoters of the present invention. Transgenic plants were regenerated on glyphosate containing media and plants rooted on a rooting media. The rooted plantlets were potted in soil and transferred to a growth chamber for a hardening off period. The seed from these plant lines were collected and planted. Fifteen plants from each line were sprayed with glyphosate at 48 ounces/acre at the 4 leaf stage. At least 8 surviving plants from each line were sprayed again at the 8 leaf stage with glyphosate at 48 ounces/acre. At maturity, the number of first position bolls for the first five bolls was counted. Those lines that had 3 or more of the first position bolls retained after the glyphosate spray (plant map.gtoreq.3) were advanced for further study. Table 7 illustrates the data produced from this study. The number of lines mapped indicates the number of lines surviving the first glyphosate spray application. The commercial standard is Line 1445 (pMON17136) that contains the P-FMV promoter driving expression of the CTP2-aroA:CP4 gene/E9 3', this line retains less than 1 of the 5 first bolls. The constructs, pCGN8099, pCGN9153, pCGN8088, pCGN8068 provided sufficient reproductive glyphosate tolerance in cotton such that 14-35% of the lines tested from these constructs were advanced for further agronomic trials.

Detailed Description Text (142):
Cotton yield is correlated with the number of squares set during the first four to five weeks of squaring. The retention of these squares to mature bolls and their contribution to the harvest of the cotton lint is a key component of yield. When determining the efficacy of transgene constructs for conferring herbicide tolerance in cotton, the amount of boll retention is a measure of efficacy and is a desirable trait. Transgenic cotton plants containing promoters of the present invention were assayed in field conditions at two locations for boll retention. The transgenic cotton lines 502-254-2 (pCGN8068), 701-178-2 (pCGN8068), 53-2 (pCGN8088), 178-1 (pCGN9153), and 60-1 (pCGN9153) were compared to 1445 (glyphosate tolerance line) and PM1218BR (Paymaster 1218 parent) that contain the construct pMON17136 (P-FMV/CTP2-aroA:CP4/E93'), a wild type non-transgenic line, Coker 130 was included. The field design is a randomized complete block design consisting of 2

rows.times.20-30 feet.times.3 replications. Glyphosate is applied as Roundup Ultra.TM. formulation at rates of 1.12 lb ai/A=48 oz product and 1.5 lb ai/A=64 oz product at the 8 leaf stage of cotton plant development. All of the cotton plots are managed aggressively for weed and insect pest control, as well as other agronomic inputs such as planting time, fertilization, irrigation, PGR usage and defoliation. The percent boll retention is determined by mapping the location of each of the retained bolls by random selection of ten plants from the middle of the two center rows (five from each row) of each plot to map. The first mapping should be done 4 weeks after first flower (mid-season map), a second mapping should be done at harvest. The data collected includes the number of first position bolls on the bottom five flowering nodes that are counted as an indication of the reproductive tolerance of the transgenic cotton lines to glyphosate. Table 8 illustrates the advantage that promoters of the present invention have conferred to transgenic cotton plants for boll retention. This enhanced reproductive tolerance has resulted in increased lint yield (Table 9) and increased seed yield (Table 10) as well.

Detailed Description Text (144):
The efficacy of the hybrid promoter P-FMV-AtEF1.alpha. driving expression of the CTP2-aroA:CP4 coding sequence (FIG. 13, pMON52059) and P-FMV/CTP2-aroA:CP4/E93' (pMON15737) was compared in transgenic Arabidopsis thaliana. The transgenic Arabidopsis thaliana plants were produced by the vacuum infiltration (Bechtold et al., C R Acad Paris Life Sci 316: 1194-1199) seeds were potted in soil in trays in a growth chamber adjusted for 24.degree. C., 16 hour light (120 .mu.E m.sup.-2 s.sup.-1) cycle to permit normal growth and development of the plants. The pMON52059 V1 event glyphosate tolerant transgenic Arabidopsis plants were selected by spray application of glyphosate herbicide at a rate of 24 ounces/acre, the surviving plants were transplanted into individual pots. Eight pMON52059 V1 plants and eight pMON15737 homozygous plants were sprayed a second time corresponding to the observation of bolting, approximately 16 days after the at a rate of 24 ounces/acre. The second spray will determine the efficacy of the two constructs for conferring reproductive tolerance. The plants were observed for vegetative effects of glyphosate application. All plants had complete vegetative tolerance and no abnormal flowers were observed. However, abortion of siliques occurred indicated that seed had not been set in the aborted siliques. The total number of siliques produced by each plant and the siliques that contained seeds (fertile siliques) were counted and tabulated. The results are shown in Table 9 and indicate that the hybrid promoter construct pMON52059 demonstrated a greater than 10 fold improvement in fertile siliques, 89% compared to pMON15737 at 8%. The number of fertile fruiting structures is related to the amount of seed that can be produced, this is especially important in crops whose yield is associated with seed numbers. Crops such as cotton, soybean, canola, wheat, and corn are crops where reproductive tolerance to glyphosate is essential for good yield.

Detailed Description Text (146):
Sunflower (Helianthus annuus L.) is a crop of agronomic importance for oil and food. The constructs pMON45325 (FIG. 2), pMON45332 (FIG. 4), and pMON45331 (FIG. 3) of the present invention were transformed into sunflower. Agrobacterium-mediated transformation of sunflower has been reported (Schrarmneijer et al., Plant Cell Reports, 9: 55-60, 1990; EP 0 486 234). Methods known by those skilled in the art of plant transformation with transgene expression constructs can include hypocotyls, apical meristems, protoplasm, and other sunflower tissues. Transgenic sunflower lines SFB250-27 contains pMON20999 (P-FMV/CTP2-aroA:CP4/E93') expression cassette; SFB288-01, SFB295-09 contain pMON45325 (P-eFMV/CTP2-aroA:CP4/E93'::P-AtAct11+intron/CTP2-aroA:CP4/E93'); SFB289-01 contain pMON45332 (P-AtEF1.alpha.+intron/CTP2-aroA:CP4/E93'::P-eFMV/CTP2-aroA:CP4/E93'); SFB303-08, SFB303-09, SFB303-11, and HA300B contain pMON45331 (P-AtEF1.alpha.+intron/CTP2-aroA:CP4/E9). These lines are tested for glyphosate tolerance and are shown in Table 12.

Detailed Description Text (148):
Vegetative glyphosate tolerant transgenic sunflower lines are scored for number of heads, precent normal heads, percent normal head size, and percent normal pollen shed. These traits are scored in a field test at one location. The tabulation of the head scores and pollen production is shown in Table 12. Lines selected from the constructs of the present invention show greater percent of normal heads, generally greater percent normal head size and better pollen shed.

09/682,769

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L14	L13 and (transgen$2 near5 probe$1)	2	L14
L13	l4 and (detect$3 near5 hybridiz$5)	39	L13
L12	L11 and PV-GHGT07	0	L12
L11	L10 and (detect$3 near5 probe$1)	32	L11
L10	l7 and transgen$2	121	L10
L9	l8 and (detect$3 near5 transgen$2)	0	L9
L8	L7 and (detect$3 near5 trensgen$2)	0	L8
L7	l4 and hybridiz$5	126	L7
L6	L5 and (hybridiz$5 near5 probe$)	0	L6
L5	L4 and (hybridz$4 near5 probe$1)	0	L5
L4	L3 and herbicide	160	L4
L3	l1 and (glyphosate near5 toleran$2)	176	L3
L2	L1 and glyphosate	1048	L2
L1	cotton$1 or gossypium hirsutum	118963	L1

END OF SEARCH HISTORY

End of Result Set

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L14: Entry 2 of 2 File: USPT May 7, 2002

DOCUMENT-IDENTIFIER: US 6384207 B1
TITLE: Regulatory sequences for transgenic plants

Brief Summary Text (11):
Despite both the important role of tissue specific promoters in plant development, and the opportunity that availability of a root preferential promoter would represent for plant biotechnology, relatively little work has yet been done on the regulation of gene expression in roots. Yamamoto reported the expression of E. coli: uidA gene, encoding .beta.-glucuronidase (GUS), under control of the promoter of a tobacco (N. tabacum) root-specific gene, TobRB7. Yamamoto et al. (1991), Conkling et al. (1990). Root specific expression of the fusion genes was analyzed in transgenic tobacco. Significant expression was found in the root-tip meristem and vascular bundle. EPO Application Number 452 269 (De Framond) teaches that promoters from metallathionein-like genes are able to function as promoters of tissue-preferential transcription of associated DNA sequences in plants, particularly in the roots. Specifically, a promoter from a metallathionein-like gene was operably linked to a GUS reporter gene and tobacco leaf disks were transformed. The promoter was shown to express in roots, leaves and stems. WO 9113992 (Croy, et al.) teaches that rape (Brassica napus L.) extensin gene promoters are capable of directing tissue-preferential transcription of associated DNA sequences in plants, particularly in the roots. Specifically, a rape extensin gene promoter was operably linked to a extA (extensin structural gene) and tobacco leaf disks were transformed. It was reported that northern analysis revealed no hybridization of an extensin probe to leaf RNA from either control or transformed tobacco plants and hybridization of the extensin probe to transgenic root RNA of all transformants tested, although the levels of hybridization varied for the transformants tested. While each of these promoters has shown some level of tissue-preferential gene expression in a dicot model system (tobacco), the specificity of these promoters, and expression patterns and levels resulting from activity of the promoters, has yet to be achieved in monocots, particularly maize.

Brief Summary Text (69):
In another of its aspects, the invention provides a transformed plant comprising at least one plant cell that contains a DNA construct of the invention. The plant may be a monocot or dicot. Preferred plants are maize, rice, cotton and tobacco.

Brief Summary Text (87):
The gene of interest may be any gene that it is desired to express in plants. Particularly useful genes are those that confer tolerance to herbicides, insects, or viruses, and genes that provide improved nutritional value or processing characteristics of the plant. Examples of suitable agronomically useful genes include the insecticidal gene from Bacillus thuringiensis for conferring insect resistance and the 5'-enolpyruvyl-3'-phosphoshikimate synthase (EPSPS) gene and any variant thereof for conferring tolerance to glyphosate herbicides. Other suitable genes are identified hereinafter. As is readily understood by those skilled in the art, any agronomically important gene conferring a desired trait can be used.

Brief Summary Text (131):
Plant cells from monocotyledonous or dicotyledonous plants can be transformed according to the present invention. Monocotyledonous species include barley, wheat, maize, oat and sorghum and rice. Dicotyledonous species include tobacco, tomato, sunflower, cotton, sugarbeet, potato, lettuce, melon, soybean and canola (rapeseed).

Detailed Description Text (18):

Using the per5 cDNA full length probe two strong hybridization signals were detected in each digest. This suggested that the per5 gene may be present in two copies per haploid genome. However, using GSP5 as a probe only one band per lane was detected which suggested that there is only one copy of the per5 gene per haploid genome and that the other hybridizing band on the genomic DNA blot corresponds to more distantly related sequences. This also demonstrated that probe GSP5 was gene specific and would be suitable for the isolation of the peroxidase genomic clone from a maize genomic library.

Detailed Description Text (59):
pDAB367 (Example 27) and pDAB419 were co-precipitated onto the surface of 1.5-3.0 rnicron gold particles (Aldrich Chem. Co., Milwaukee, Wis.). pDAB367 contains a phosphinothricin acetyl transferase gene fusion which encodes resistance to the herbicide Basta..TM. This gene is used to select stable transgenic events. The precipitation mixture included 60 mg of pre-washed gold particles, 140 .mu.g of plasmid DNA (70 .mu.g of each) in 300 .mu.L of sterile water, 74 .mu.L of 2.5 M CaCl.sub.2, and 30 .mu.L of 0.1 M spermidine. After adding the components in the above order, the mixture was vortexed immediately, and allowed to settle for 2-3 minutes. The supernatant was removed and discarded and the plasmid/gold particles were resuspended in 1 mL of 100% ethanol and diluted to 7.5 mg plasmid/gold particles per mL of ethanol just prior to blasting.

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L14: Entry 1 of 2 File: USPT Jan 14, 2003

DOCUMENT-IDENTIFIER: US 6506962 B1
TITLE: Acquired resistance genes in plants

Detailed Description Text (74):
Useful inducible promoters include promoters induced by salicylic acid or polyacrylic acids (PR-1, Williams et al., 1992), or SA analogs, such as 2,6-dichloroisonicotinic acid (INA) or benzo(1,2,3) thiodiazole-7-carbothioic acid S-methyl ester (BTH) (Gorlach et al., 1996; Kessman et al., 1994), induced by application of safeners (substituted benzenesulfonamide herbicides, Hershey and Stoner, 1991), heat-shock promoters (Ou-Lee et al., 1986; Ainley et al., 1990), a nitrate-inducible promoter derived from the spinach nitrite reductase sequence (Back et al., 1991), hormone-inducible promoters (Yamaguchi-Shinozaki et al., 1990; Kares et al., 1990), the WCI-3 promoter, and light-inducible promoters associated with the small subunit of RuBP carboxylase and LHCP families (Kuhlemeier et al., 1989; Feinbaum et al., 1991; Weisshaar et al., 1991; Lam and Chua, 1990; Castresana et al., 1988; Schulze-Lefert et al., 1989).

Detailed Description Text (92):
The recombinant vector may further comprise a selectable marker. The nucleic acid sequence serving as the selectable marker functions to produce a phenotype in cells that facilitates their identification relative to cells not containing the marker. Useful selectable markers include GUS, green fluorescent protein (GFP), neomycin phosphotransferase II (nptII), luciferase (LUX), chloramphenicol acetyl transferase (CAT), antibiotic resistance sequences, and herbicide (e.g., glyphosate) tolerance sequences. The selectable marker is preferably a kanamycin, hygromycin, or herbicide resistance marker.

Detailed Description Text (96):
Short nucleic acid sequences having the ability to specifically hybridize to complementary nucleic acid sequences may be produced and utilized in the present invention. These short nucleic acid molecules may be used as probes to identify the presence of a complementary sequence in a given sample. Thus, by constructing a nucleic acid probe that is complementary to a small portion of a particular nucleic acid sequence, the presence of that sequence may be assessed. Use of these probes may greatly facilitate the identification of transgenic plants that contain a particular nucleic acid sequence (e.g., a nucleic acid sequence encoding an acquired resistance gene). The probes may also be used to screen cDNA or genomic libraries for additional sequences encoding acquired resistance genes.

Detailed Description Text (110):
The transformed host cell may generally be any cell that is compatible with the present invention. The transformed host cell preferably is prokaryotic, such as a bacterial cell, and more preferably is an Agrobacterium, Arthrobacter, Azospyrillum, Clavibacter, Escherichia, Pseudomonas, or Rhizobacterium cell. The transformed host cell preferably is eukaryotic, and more preferably is a plant, yeast, or fungal cell. If a yeast cell is selected to be transformed, it preferably is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, or Pichia pastoris. If a plant cell is selected to be transformed, it may be of any type capable of being transformed, preferably one with an agronomic, horticultural, ornamental, economic, or commercial value, and more preferably is an Acacia, alfalfa, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, broccoli, brussels sprouts, cabbage, canola, cantaloupe, carrot, cassava, castorbean, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, clover, coconut, coffee, corn, cotton, cucumber, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, figs, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, linseed, mango, melon, mushroom, nectarine, nut,

oat, oil palm, oil seed rape, okra, olive, onion, orange, an ornamental plant, palm, papaya, parsley, parsnip, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, tangerine, tea, tobacco, tomato, triticale, turf, turnip, a vine, watermelon, wheat, yams, and zucchini cell.

Detailed Description Text (148):
The gene copy number of Nph homologs in monocot species was examined using monocot-specific probes in Southern blot analysis. The cloned wheat Nph2-1 cDNA (SEQ ID NO:7) in pBluescript SK+ plasmid (Stratagene; 30 cycles of PCR: 94.degree. C. 5 min/94.degree. C. 1 min/55.degree. C. 1 min /72.degree. C. 1.5 min/72.degree. C. 10 min at cycle 30) was amplified using KS and SK primers (Stratagene, La Jolla, Calif.) to generate the Nph2 probe. Genomic DNA isolated from wheat (cv. Bobwhite), barley (cv. Perry), corn (cv. B-73), and rice (cv. M202) was digested with either EcoRI or HinDIII restriction enzyme, fragments were separated on agarose gels, transferred to a HYBOND N+ nylon filter (Amersham Life Sciences, Inc., Arlington Heights, Ill.), and incubated with the wheat Nph2-1 probe prepared by .sup.32 P random priming. Southern hybridization was performed overnight at 65.degree. C. using 20 mL Rapid-Hyb buffer (Amersham Life Science, Inc., Arlington Heights, Ill.) with 100 .mu.g/mL fish sperm DNA. Filters were washed twice at 2.times.SSC/0.1% SDS (65.degree. C. , 20 min) and twice at 0.5.times.SSC/0.1% SDS (65.degree. C. ,20 min.). Hybridizing bands were detected by autoradiography.